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Preliminary clinical application of a mathematical model for interpreting dehydroepiandrosterone-sulfate loading test in late pregnancy

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In 1967, LAURITZEN proposed a dynamic test to investigate placental steroidogenesis [7]. Intravenous injection of 50 mg dehydroepiandrosterone-sulfate (DHEA-S) to pregnant women induced an increase of urinary estriol excretion; but with placental insufficiency this increase was absent or reduced. Since 1973 there have been several studies of the DHEA-S Loading Test (DLT) in which maternal plasma levels of estrogens were determined [1, 2, 3, 4, 5, 6, 8, 12, 13, 14]. After an intravenous injection of 50 mg of DHEA-S, Estrone (E_1) levels increase about threefold within two to four hours and return to baseline values eight to twelve hours later; Estradiol (E_2) levels increase two to fivefold within the first hour and return to initial values twelve hours later; unconjugated estriol (E_3) sometimes shows an increase of 50% between six and twelve hours after the injection [13]. An adequate method for interpreting the DLT in clinical use has not yet been found. The accuracy of the plasmatic DLT to indicate fetal risk has not been proven with certainty. Nevertheless NAGEY and PUPKINS [9, 11] using a mathematical representation of a physiological model of the DHEA to estrogen conversion system, demonstrated the ability of DLT to indicate fetal jeopardy or/and placental insufficiency. Using a similar model, we have attempted to investigate the decrease of placental aromatization in intra-uterine fetal growth retardation.

Curriculum vitae

HENRY JACQUES THOUMSIN *was born in Belgian Congo (now Zaïre) on April 19th, 1943. In 1969 he graduated in medicine at the University of Liège, Belgium. He trained in the department of Obstetrics and Gynecology of the University of Liège under Prof. Dr.* R. LAMBOTTE *and in 1974 obtained his Belgian Specialist Board qualification as gynecologist and obstetrician. Since then he has been in charge of the prenatal care unit of the University of Liège and is leading intensive research in feto-placental steroidogenesis. His fields of interest include fetoplacental physiopathology, prenatal and intrapartal care, poor intrauterine fetal growth and prematurity, hormonology (estrogens), cardiotocography and tocolysis.*

1 Material and method

A Single DLT was performed on each of 27 pregnant women at 32–42 weeks gestation. All the patients were under care from the beginning of pregnancy till delivery by the Obstetrical department of the University of Liege. There was no fetal or neonatal death in the study. An intravenous injection of 50 mg DHEA-S (Ro 6-6827/6: ROCHE S. A./Basel) was given in a single bolus at eight o'clock in the morning. A blood sample was collect-

ed prior to the injection and at least five samples in the following three hours, the first one no more than 30 minutes after the injection. Serum unconjugated E_2 was determined by radioimmunoassay after extraction with ether and eluation on sephadex LH-20 columns in a benzene-dichloromethane-methanol system (60v–30v–10v). **The results were analyzed by fitting a mathematical representation of a physiologic model of the DHEA-S to E_2 conversion system, using a computer program.** This model is a modification of NAGEY's [9]. It allows the evaluation of the DHEA-S to E_2 conversion rate constant given the serum E_2 concentrations and the time intervals after injection. Details on biological facts and assumptions and on the mathematical development which lead to the establishment of the model are clearly explained in NAGEY's publication. Briefly, DHEA-S to E_2 conversion rate constant is calculated by fitting the following equation to the DLT data from each patient:

$$E_2(t) = E_2(o) + \frac{b\,Qo}{a+b+c}\left(e^{-ct} - e^{-(a+b)t}\right)$$

where: t = time interval (min)

$E_2(t)$ = Estradiol concentrations at different times after injection (Moles/litre)

$E_2(o)$ = Estradiol concentration before injection (Moles/litre)

Qo = DHEA-S concentration change induced by the DHEA-S injection = quantity of DHEA-S injected divided by plasma volume (Moles/litre)

a = rate constant of DHEA-S to non-E_2 clearance

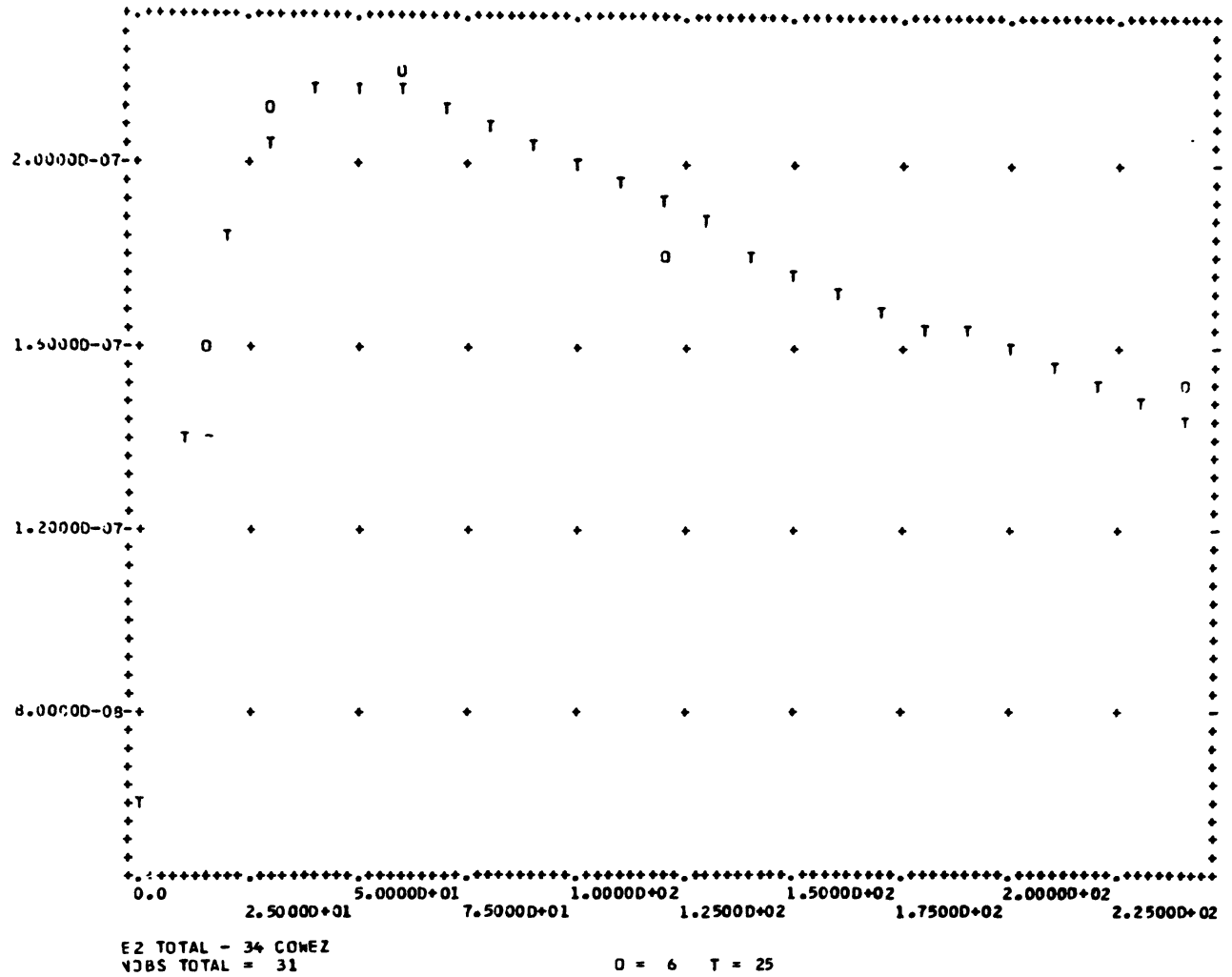

Fig. 1. An example of the course of E_2 levels (Moles/litre) given by the computer in case n° 34 (o = DLT datas; T = calculated points; T and o values are indentical at time 0 and 180.
E_2 level scale: from 4.10^{-8} to $2{,}4.10^{-7}$ Moles/litre (i.e. 1.2000 D – 07 = $1{,}2.10^{-7}$)
time scale: from 0 to 225 minutes (i.e.: 1.2500 D + 02 = $1{,}25.10^{-2}$ = 125)

Tab. I. Patients included in the study and results of DLT

Patient n°	gest. age at test time (wk)	pregnancy complication	gest. age at birth (wk)	fetal or placental impairment	E_2 (ng/ml) baseline value	DS to E_2 conversion rate constant ($x10^{-6}$ min^{-1})
	group I					
1	35	none	41	none	17	241
4	40	none	40	none	16	1201
10	34	none	40	none	18	259
45	38	none	40	none	31	4115
47	35	none	41	none	16	128
48	32	none	37	none	12	2152
51	39	none	39	none	40	533
52	38	none	39	none	25	758
	group II					
3	30	premature labor	34	none	12	80
35	35	premature labor	35	none	4	140
44	35	premature labor	37	none	23	266
	group III					
2	38	very low urinary E_3	38	none	8	140
5	39	diabetes-a	39	none	8	168
9	37	nephrotic syndrome	40	none	8	2547
40	31	pyelonephritis	38	none	4	80
43	37	diabetes-a	40	none (plac. sclerosis)	6	133
72	37	hyperandrogenism mild toxemia dexamethazone treatment	42	none	7	3554
	group IV					
8	42	post-term	42	none	40	66
38	41	post-term	42	none	4	102
39	42	post-term	43	acute fetal distress	6	11
	group V					
34	35	severe toxemia	38	IUGR	7	172
41	35	none (history of IUFD)	37	IUGR	5	11
42	35	cardiopathy	39	IUGR	4	88
68	35	severe toxemia	36	IUGR acute fetal distress	5	260
69	39	anemia (thalassemia)	39	IUGR	5	247
70	40	none	40	IUGR	5	157
	others					
46	38	none	41	hydrocephaly	22	2254

b = rate constant of DHEA-S to E_2 conversion (Min^{-1})

c = rate constant of E_2 clearance

Estimation of parameters is based on the least squares method. Owing to the fact that the relation between E_2 and parameters is a non-linear one, the problem does not admit analytical solutions; parameters have been evaluated in all cases, using a computer program of function minimization based on the NELDER-MEAD simplex method [10].

Fig. 1. shows an example of the course of E_2 levels given by the computer when fitting the equation to the DLT data of a representative patient (n° 34).

The constant b, which reflects placental aromatization activity, is the required parameter.

To clarify the meaning of b, it can be said that it represents the proportion of exogenous DHEA-S which can be converted to E_2 by the placenta in one minute.

2 Results

Tab. I gives the results of the DLTs in the 27 patients divided into six groups according to maternal pathology or pregnancy complication and feto-placental outcome.

Group I is the control group and comprises eight patients who had uneventful pregnancies and normally grown undistressed infants.

Group II comprises three cases of premature labor without any fetal impairment.

Group III contains six cases of various pathological situations at test time without any feto-placental complications. Group IV includes three post-term DLT. One fetus presented acute distress during labor.

Group V is composed of six cases of intra-uterine fetal growth retardation (I.U.G.R.) in which birth weights were below the 10th percentile. Details on pregnancy complication is given in the table.

Group VI is a case of sex-linked hydrocephaly.

Basal E_2 levels (ng/ml) and DHEA-S to E_2 conversion rate constant ($min^{-1} \times 10^{-6}$) are written for each DLT.

Tab. II presents the mean and range (after logarithmic transformation) of basal E_2 values and of DHEA-S to E_2 conversion rate constants for the three main clinical groups: Control group (I); pathological pregnancy group without fetal impairment (III) and I.U.G.R. group (V).

Tab. II. Comparisons between group I and groups III and V for serum E_2 concentrations and for DHEA-S to E_2 conversion rate constant (*: statistically significant at probability level $\alpha = 0.05$)

	group III	group I (control)	group V
E_2 baseline value (ng/ml) (mean (°) and range)	6.6* (4.0→8.0)	20.0 (12.0→40.0)	5.1* (4.0→7.0)
DS to E_2 conversion rate constant ($X10^{-6}$ min^{-1}) (mean (°) and range)	363 NS (80→3554)	656 (128→4115)	109* (11→260)

(°) (The means, expressed in original units, are calculated from logarithms of the observation).

Statistical comparison between group I and III and between group I and V uses the non-parametric confidence interval method. Serum E_2 concentrations before precursor injection are significantly lower in both the pathological pregnancy group and the I.U.G.R. group. DHEA-S to E_2 conversion rate constants are not significantly reduced in group III but are markedly impaired in group V.

3 Comments

It seems probable that in the pathogenesis of fetal risk, as found in intra-uterine growth retardation, the decrease of utero-placental perfusion is usually the first event to occur. This decrease of utero-placental blood flow occurs in various disturbances of pregnancy but acquires clinical importance when leading to fetal distress. It can be presumed that the decrease of blood flow, when sufficient to induce poor fetal growth may also lead to a diminution of placental enzyme capacity.

This diminution cannot be demonstrated by simple blood estrogen determination which may be reduced by a low availability of precursors. Many patients excrete little estrogen without lowered placental aromatization. **It appears of first importance to evaluate the placental biosynthetic capacity as an information on fetal health.**

The DHEA-S to E_2 conversion rate constant, such as determined in this paper, reflects the speed of placental aromatization of C-19 precursors to estrogens. It may thus be considered as a good index of placental enzyme capacity. **It is shown that a low DHEA-S to E_2 conversion rate constant is more often associated with lowered fetal growth which cannot be indicated by simple basal E_2 determination.** The low estrogen levels in fetal impairment do not result from a diminution of precursor availability alone but also from reduced placental enzyme capacity.

This mathematical model is not applicable to serum unconjugated E_3 concentrations for two reasons. First, the E_3 rise after i.v. DHEA-S injection is not always significant and occurs more than four hours after the injection. Thus one of the assumptions which lead to the establishment of the model is invalid in this case [9].

Secondly, E_3 production from C-19 precursors involves a 16-α hydroxylation which mainly occurs in the fetus or in the maternal liver. The physiological model considered simply as a two compartments model is barely sufficient to describe the E_2 change during the DLT. The model should certainly be inadequate for investigating three or more compartments as for E_3.

As described in this paper, **DLT using the calculation of the DHEA-S to E_2 conversion rate constant allows only a placental evaluation.** But this evaluation is more sensitive because by administering DHEA-S the unknown variable "precursor" is excluded. We agree with LAURITZEN's opinion [8] that the test is not a substitute for the usual estrogen determinations, but should rather be complementary to them and give additional information on the eventual repercussions of the so called "placental insufficiency" on fetal well-being.

Summary

This study attempts to investigate the decrease of placental aromatization of dehydroepiandrosterone-sulfate (DHEA-S) to estrogens, in case of fetal growth retardation.

27 intravenous DHEA-S loading tests (DLT) have been performed in 27 pregnant women (Tab. I): There were eight normal pregnant women with normal babies (group I, control); six cases of various pathological situations without fetal impairment (group III), and six cases of intra-uterine fetal growth retardation (group V, I.U.G.R.). The dose of DHEA-S was 50 mg. The parameter investigated was the maternal serum concentration of Estradiol (E_2) within the first three hours following the injection. The data were analyzed by fitting a mathematical representation of DHEA-S to E_2 conversion system using a computer program. **This mathematical model permits the calculation of the DHEA-S to E_2 conversion rate constant, which represents the proportion of exogenous DHEA-S converted in E_2 in one minute by the placenta and thus reflects the placental ability to aromatize C19 precursors.** Table II presents the mean and range of E_2 baseline values and of DHEA-S to E_2 conversion rate constants for the three defined groups. **Statistical comparison shows that E_2 serum concentrations before the test are lower in both pathological group (III) and IUGR group (V) while DHEA-S to E_2 conversion rate constant is impaired only in the IUGR group (V).** It is concluded that fetal growth retardation, resulting from placental insufficiency, is more often associated with a lack of placental steroidosynthesis. The lowered estrogen excretion in case of fetal impairment does not result from a diminution of precursor availability alone but also from a reduced placental enzymatic capacity. The DLT and the calculation of DHEA-S to E_2 conversion rate constant is able to indicate the enzymatic insufficiency.

It constitutes a sensitive test for evaluating placental disturbance.

Keywords: DHEA-S (loading test), estradiol metabolism, fetal growth retardation, placenta insufficiency, placenta metabolism.

Zusammenfassung

Klinische Anwendung eines mathematischen Modells zur Interpretation des Dehydroepiandrosteron-Belastungstestes im letzten Trimester der Schwangerschaft.

In dem vorliegenden Artikel wird die Abnahme von Östrogen bei verzögertem Wachstum des Foeten unter Verwendung von Dehydroepiandrosterone Sulfat (DHEA-S) untersucht.

Es wurden 27 DHEA-S Belastungstests (nach LAURITZEN) durchgeführt (Tab. I). 8 Schwangerschaften waren normal, 6 pathologisch mit normalem Fötus und in weiteren 6 verlief das Wachstum des Foeten verzögert.

In allen Fällen wurden 50 mg DHEA-S injiziert und die Zunahme an Östradiol (E_2) im Plasma mehrmals innerhalb drei Stunden nach der Einspritzung gemessen. Die Analyse der Werte erfolgte nach einem mathematischen Modell mit Hilfe eines Computers. Das Modell zeigt die Biotransformation von E_2 aus DHEA-S in der Plazenta. **Mit diesem Modell kann die Umwandlungskonstante (DHEA-S in E_2), das heißt das Verhältnis von DHEA-S zu E_2 errechnet werden.** Sie spiegelt die Fähigkeit der Plazenta, C19-Steroide in Östrogene umzuwandeln, wieder.

Tab. II zeigt die mittleren und die Extremwerte der Blutöstrogene und die Umwandlungskonstante (DHEA-S in E_2) der drei oben genannten Gruppen.

Die statistische Untersuchung zeigt, daß die Blutöstro-

gene bei pathologischen Schwangerschaften und bei verzögertem Wachstum des Foeten erniedrigt sind, während die Umwandlungskonstante nur in Fällen von verzögertem Wachstum des Foeten erniedrigt ist.
Hieraus läßt sich folgendes schließen: Mangelndes Foetalwachstum, das durch plazentare Insuffizienz verursacht wurde, ist häufig an einen Mangel von plazentarer Steroidsynthese gekoppelt. Die Schwäche der Steroidsynthese erklärt auch die erniedrigten Blutöstrogene.

Der DHEA-S-Test und die Errechnung der Umwandlungskonstante (DHEA-S in E_2) mit Hilfe unserer mathematischen Formel stellt eine gute Möglichkeit dar, das metabolische Fehlverhalten in der Plazenta zu untersuchen.

Schlüsselworte: DHEA-S-Test, Östrogene, Plazentainsuffizienz, Wachstumsretardierung.

Résumé

Application clinique préliminaire d'un modèle mathématique d'interprétation du test de surcharge au sulfate de déhydroépiandrostérone au dernier trimestre de la grossesse.
L'étude envisage le problème d'une diminution de l'aromatisation du sulfate de déhydroépiandrostérone (DHEA-S) en oestrogènes dans les retards de croissance foetale intra-utérine.
27 tests de surcharge intra-veineuse au DHEA-S ont été réalisés chez 27 gestantes parmi lesquelles (Tab. I): huit gestantes normales ayant eu des enfants normaux (groupe I, contrôle); six cas de pathologie gestationnelle sans répercussion foetale (groupe III) et six cas de retard de croissance intra-utérine (groupe V, I.U.G.R.). La dose de DHEA-S était de 50 mg dans tous les cas. Le paramètre étudié fut la concentration sérique maternelle en oestradiol (E_2) mesurée à plusieurs reprises endéans les trois heures suivant l'injection. Les données sont analysées sur ordinateur au moyen d'un modèle mathématique censé représenter le plus exactement possible le système de conversion du DHEA-S en oestrogène dans le placenta.
Ce modèle permet le calcul de la constante de conversion du DHEA-S en E_2 qui reprirente la proportion du DHEA-S transformée en E_2 en une minute. C'est un reflet de la capacité placentaire d'aromatisation des précurseurs en C-19 en oestrogènes.

La table II présente les valeurs moyennes et extrêmes des taux de base en E_2 et des constantes de conversion du DHEA-S en E_2 enregistrées dans les trois groupes définis plus haut.

L'étude statistique montre que les taux de E_2 sont abaissés tant dans les grossesses pathologiques (Groupe III) que dans les retards de croissance intra-utérine (groupe V I'U'G'R') tandis que la constante de conversion du DHEA-S en E_2 n'est diminuée que dans le cadre du retard de croissance (groupe V).

En conclusion, le retard de croissance résultant d'une insuffisance placentaire est bel et bien associé à un défaut d'aromatisation placentaire qui contribue à expliquer en partie les taux circulant bas en oestrogènes.
Le test au DHEA-S et le calcul de la constante de conversion du DHEA-S en E_2 par la formule mathématique autorise une approche très fine des troubles métaboliques placentaires.

Mots clés: Croissance foetale, DHEA-test, insuffisance placentaire, métabolisme placentaire, Oestradiol.

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